

REMARKS

Claims 1-19 are pending and under consideration in the instant application. With this amendment, the specification and Claims 1 and 5 have been amended. Following entry of the present amendment, Claims 1-19 are pending and under consideration. A version with markings to show changes made to the specification is attached as *Appendix A*. A clean version of the amended paragraphs of the specification is attached as *Appendix B*. A version with markings to show changes made to Claim 1 is attached as *Appendix C*. Finally, a clean version of the claims pending following entry of the present amendment is attached as *Appendix D*.

I. THE AMENDMENT OF THE SPECIFICATION

The specification has been amended on page 13, lines 10 to 28; page 19, line 21 to page 21, line 12; page 25, lines 7 to 18; and page 41, lines 13 to 16, while the abstract has been amended on page 60, lines 3 to 17. These amendments describe that one primer binds to a first binding sequence A' which is complementary to a sequence A and the other primer binds to a second binding sequence C which is located in the 3' direction from A and does not overlap A. The amendments of the specification are supported by the specification, for example, at page 49, lines 1 to 12, and at page 54, lines 4 to 7 and by Figures 2, 5, 6, 7, and 8.

For example, Figure 2 depicts the amplificate as comprising sequences A, B and C, wherein C is located in the 3' direction of A. Also depicted is the complement of the amplificate comprising sequences A', B' and C'. To produce such an amplificate, the primers must bind to sequences A' and C rather than A and C'. The Examples provided in the specification, and Figures 5, 6, 7, and 8 demonstrate the correct directionality and binding of primers that bind sequences corresponding to sequences A and C'. For instance, the assay on page 49 provides examples of primers that were used to amplify regions of the HCV genome. The forward primer (HC2F) is essentially identical to the sequence at the 5' region of the amplificate, while the reverse primer (HC1F-bio) is complementary to the sequence at the 3' region of the amplificate.

In addition, Figures 6 and 7 provide a section of the HCV genome from positions 261 to 333 in the 5' to 3' direction and multiple primer pairs that can be used to amplify this region of the HCV genome. In each case, the forward primer (for example, CK10) is essentially identical to the sequence at the 5' region and the reverse primer (for example, CK20) is complementary to the sequence at the 3' region. The forward primer, CK10, binds to the complementary strand of the HCV strand provided, corresponding to binding sequence

A'. The reverse primer, CK20, binds to binding sequence C, which is in the 3' direction from A. Thus, Figures 6 and 7 provide support for the amendments to the specification.

Furthermore, Figures 5 and 8 provide additional examples of forward and reverse primers that can be used to amplify regions of the HCV genome. Each forward primer corresponds to the sequence at the 5' region of the amplicate, thereby binding the complement to this sequence, corresponding to region A', while the reverse primers bind to the 3' end of the amplicate, corresponding to region C. Accordingly, Figures 5 and 8 provide additional support for the amendment to the specification.

Given the support in the specification, including the examples and figures, the amendments to the specification do not introduce new matter. Accordingly, entry of the amendments to the specification under 37 C.F.R § 1.111 is hereby respectfully requested.

II. THE AMENDMENT OF THE CLAIMS

Claim 1 has been amended to recite a method for the detection of a nucleic acid comprising (a)- producing a plurality of amplicates of a section of the nucleic acid with the aid of two primers, one of which can bind to a binding sequence A', which is essentially complementary to a sequence A of one strand of the nucleic acid, and the other can bind to a binding sequence C which is located in the 3' direction from A and does not overlap A; and (b)- contacting the amplicates with a probe having a binding sequence D which can bind to a sequence B located between the sequences A and C or to the complement thereof; and (c)- detecting the formation of a hybrid of the amplicate and probe, wherein the sequence between the binding sequences A and C contains no nucleotides that do not belong to a sequence E of the amplicate that is bound by binding sequence D of the probe and the amplicate does not exceed a total length of 100 nucleotides.

Support for amended Claim 1 can be found in the specification, for example, at page 49, lines 1 to 12, and at page 54, lines 4 to 7; in Figures 2, 5, 6, 7, and 8; and in Claim 1 as originally filed. Figure 2 depicts the amplicate and complement of the amplicate that is produced by primers that bind to sequences A' and C. In addition, the specification at page 49, lines 1 to 12 and Figures 6 and 7 further support amended Claim 1 by describing the HCV genomic sequence and amplicon formed therefrom, along with primers for use in the examples. Thus, Applicants respectfully submit that the amendment to Claim 1 does not introduce new matter.

Claim 5 has been amended to correct a typographical error presented in the first preliminary amendment, filed together with the instant application on May 4, 2000. Support for the amendment to Claim 5 may be found, for example, in the specification at page 19,

lines 28-32. Therefore, Applicants respectfully submit that the amendment to Claim 5 does not constitute new matter.

Accordingly, Applicants hereby respectfully request entry of the present amendment to the claims under 37 C.F.R. § 1.111.

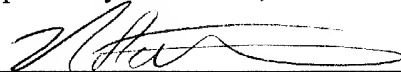
CONCLUSION

Applicants respectfully submit that Claims 1-19 satisfy all of the criteria for patentability and are in condition for allowance. An early indication of the same and passage of Claims 1-19 to issuance is therefore kindly solicited.

No fees is believed due in connection with this amendment. However, the Commissioner is authorized to charge all required fees, fees under 37 CFR § 1.17 and all required extension of time fees, or credit any overpayment, to Pennie & Edmonds LLP U.S. Deposit Account No. 16-1150.

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Respectfully submitted,

42,983

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APPENDIX A

Marked-up Version Showing Amendments to Specification

The paragraph on page 13, lines 10 to 28:

The invention concerns a method for the production of a plurality of amplicates of a section of this nucleic acid with the aid of two primers, one of which can bind to a first binding sequence [A] A', which is essentially complementary to a sequence A of a strand of the nucleic acid, and the other can bind to a second binding sequence C [C' which is complementary to a sequence C] which is located in the 3' direction from A and does not overlap A, contacting the amplicates with a probe having a binding sequence D which can bind to a third sequence (B) located between the sequences A and C or to the complement (B') thereof, and detecting the formation of a hybrid of an amplicate and the probe wherein the third sequence (B) located between the binding sequences A and C or the complement (B') thereof contains no nucleotides that are not part of the sequence section E formed from the binding sequence D of the probe and the sequence of the amplicate bound thereto. The length of the probe is preferably of the same size or larger than the sequence B or the complement B'.

Please replace the paragraph on page 19, line 21 to page 21, line 12 with the following paragraph:

In the first essential step of the method according to the invention a segment of the nucleic acid to be detected is amplified. This segment is also referred to as an amplicon in the following. It is essential that this contains the sequence region between the outer ends of the binding sequences [A and C] A' and C [or of the complement thereof] of the primers (the primer binding regions) and contains the binding region E of the probe or of the complement thereof. According to the present invention the amplicon (preferably the total length of the sequences of the regions A, B and C) is preferably shorter than 100 nucleotides, particularly preferably shorter than 60 nucleotides, but preferably longer than 40 nucleotides. However, this does not mean that the total length of the amplicates cannot be larger e.g. when the primers have additional nucleotides. Amplification methods are used which allow an amplification of the nucleic acid to be detected or the complement thereof and result in the formation of tripartite mini-nucleic acid amplification products. In principle all nucleic acid amplification methods that are known in the prior art can be used for this. Target-specific nucleic acid amplification reactions are preferably used. Theoretically exponential target-

specific nucleic acid amplification reactions are particularly preferably used in which an anti-parallel replication of the nucleic acid to be detected or of its complement is carried out e.g. elongation-based reactions such as the polymerase chain reaction (PCR for deoxyribonucleic acids, RT-PCR for ribonucleic acids) or transcription-based reactions such as e.g. nucleic acid sequence based amplification (NASBA) or transcription mediated amplification (TMA). Thermocyclic exponential elongation-based nucleic acid amplification reactions are particularly preferred such as e.g. the polymerase chain reaction. The nucleic acids to be detected or complements thereof which are used for the amplification can be present in the form of single-stranded or double-stranded deoxyribonucleic acids or ribonucleic acids. The aim of the amplification reaction (amplification) is to produce numerous amplicates of a segment of the nucleic acid to be detected. Hence an amplicate is understood as any molecular species produced by using sequence information of the nucleic acid. In particular the term refers to nucleic acids. The term "amplicate" includes single-stranded as well as double-stranded nucleic acids. In addition to the regions containing the sequence information of the underlying nucleic acid (amplicon), an amplicate can also contain additional regions which are not directly related to sequences of the nucleic acid to be amplified that are outside the ends of the primer binding sites which face away from another. Such sequences with a length of more than 15 nucleotides preferably do not occur on the nucleic acid to be detected or its complement and cannot hybridize with it by direct base pairing. Hence amplicates can either hybridize with the nucleic acid to be detected itself or with its complement. Amplicates are for example also products of an asymmetric amplification i.e. an amplification in which the two strands are formed in different amounts (e.g. by using different amounts of primers) or in which one of the two strands is subsequently destroyed (e.g. by RNase).

Please replace the paragraph on page 25, lines 7 to 18 with the following paragraph:

In the present invention the segment of the nucleic acid from which it is intended to produce a plurality of amplicates is selected such that it contains three regions A, B and C. Regions A and C are regions selected such that one primer can use the complement of sequence A as the binding sequence and [the complement of] the region C can serve as the binding sequence for the other primer. A complement within the sense of the present invention is understood as a nucleic acid or nucleic acid sequence which is essentially complementary to a certain other nucleic acid e.g. a sequence region e.g. of an amplicate or of the nucleic acid to be detected.

Please replace the paragraph on page 41, lines 13 to 16 with the following paragraph:

The primers preferably bind to the binding sequences A' or C [A or C] as described above and the probe preferably binds to a region B located between the ends of the binding sequences A' and C [A or C] or to the complement thereof.

Please replace the abstract paragraph on page 60, lines 3 to 17 with the following paragraph:

Method for the detection of a nucleic acid comprising the production of a plurality of amplicates of a section of this nucleic acid with the aid of two primers, one of which can bind to a binding sequence [A] A', which is essentially complementary to a sequence A of the nucleic acid, and the other can bind to a binding sequence C [C' which is complementary to C] which is located in the 3' direction from A and does not overlap with A, contacting the amplicates with a probe having a binding sequence D which can bind to a sequence B which is located between the sequences A and C or to the complement thereof, and detecting the formation of a hybrid of the amplicate and probe where the sequence located between the binding sequences A and C contains no nucleotides that do not belong to the binding sequence D of the probe or its complement D'.

APPENDIX B

Clean Version of Amended Paragraphs of the Specification

The paragraph on page 13, lines 10 to 28:

The invention concerns a method for the production of a plurality of amplificates of a section of this nucleic acid with the aid of two primers, one of which can bind to a first binding sequence A', which is essentially complementary to a sequence A of a strand of the nucleic acid, and the other can bind to a second binding sequence C which is located in the 3' direction from A and does not overlap A, contacting the amplificates with a probe having a binding sequence D which can bind to a third sequence (B) located between the sequences A and C or to the complement (B') thereof, and detecting the formation of a hybrid of an amplificate and the probe wherein the third sequence (B) located between the binding sequences A and C or the complement (B') thereof contains no nucleotides that are not part of the sequence section E formed from the binding sequence D of the probe and the sequence of the amplificate bound thereto. The length of the probe is preferably of the same size or larger than the sequence B or the complement B'.

The paragraph on page 19, line 21 to page 21, line 12:

In the first essential step of the method according to the invention a segment of the nucleic acid to be detected is amplified. This segment is also referred to as an amplicon in the following. It is essential that this contains the sequence region between the outer ends of the binding sequences A' and C of the primers (the primer binding regions) and contains the binding region E of the probe or of the complement thereof. According to the present invention the amplicon (preferably the total length of the sequences of the regions A, B and C) is preferably shorter than 100 nucleotides, particularly preferably shorter than 60 nucleotides, but preferably longer than 40 nucleotides. However, this does not mean that the total length of the amplificates cannot be larger e.g. when the primers have additional nucleotides. Amplification methods are used which allow an amplification of the nucleic acid to be detected or the complement thereof and result in the formation of tripartite mini-nucleic acid amplification products. In principle all nucleic acid amplification methods that are known in the prior art can be used for this. Target-specific nucleic acid amplification reactions are preferably used. Theoretically exponential target-specific nucleic acid amplification reactions are particularly preferably used in which an anti-parallel replication of the nucleic acid to be detected or of its complement is carried out e.g. elongation-based

reactions such as the polymerase chain reaction (PCR for deoxyribonucleic acids, RT-PCR for ribonucleic acids) or transcription-based reactions such as e.g. nucleic acid sequence based amplification (NASBA) or transcription mediated amplification (TMA). Thermocyclic exponential elongation-based nucleic acid amplification reactions are particularly preferred such as e.g. the polymerase chain reaction. The nucleic acids to be detected or complements thereof which are used for the amplification can be present in the form of single-stranded or double-stranded deoxyribonucleic acids or ribonucleic acids. The aim of the amplification reaction (amplification) is to produce numerous amplicates of a segment of the nucleic acid to be detected. Hence an amplicate is understood as any molecular species produced by using sequence information of the nucleic acid. In particular the term refers to nucleic acids. The term "amplicate" includes single-stranded as well as double-stranded nucleic acids. In addition to the regions containing the sequence information of the underlying nucleic acid (amplicon), an amplicate can also contain additional regions which are not directly related to sequences of the nucleic acid to be amplified that are outside the ends of the primer binding sites which face away from another. Such sequences with a length of more than 15 nucleotides preferably do not occur on the nucleic acid to be detected or its complement and cannot hybridize with it by direct base pairing. Hence amplicates can either hybridize with the nucleic acid to be detected itself or with its complement. Amplicates are for example also products of an asymmetric amplification i.e. an amplification in which the two strands are formed in different amounts (e.g. by using different amounts of primers) or in which one of the two strands is subsequently destroyed (e.g. by RNase).

The paragraph on page 25, lines 7 to 18:

In the present invention the segment of the nucleic acid from which it is intended to produce a plurality of amplicates is selected such that it contains three regions A, B and C. Regions A and C are regions selected such that one primer can use the complement of sequence A as the binding sequence and the region C can serve as the binding sequence for the other primer. A complement within the sense of the present invention is understood as a nucleic acid or nucleic acid sequence which is essentially complementary to a certain other nucleic acid e.g. a sequence region e.g. of an amplicate or of the nucleic acid to be detected.

The paragraph on page 41, lines 13 to 16:

The primers preferably bind to the binding sequences A' or C as described above and the probe preferably binds to a region B located between the ends of the binding sequences A' and C or to the complement thereof.

The abstract paragraph on page 60, lines 3 to 17:

Method for the detection of a nucleic acid comprising the production of a plurality of amplicates of a section of this nucleic acid with the aid of two primers, one of which can bind to a binding sequence A', which is essentially complementary to a sequence A of the nucleic acid, and the other can bind to a binding sequence C which is located in the 3' direction from A and does not overlap with A, contacting the amplicates with a probe having a binding sequence D which can bind to a sequence B which is located between the sequences A and C or to the complement thereof, and detecting the formation of a hybrid of the amplicate and probe where the sequence located between the binding sequences A and C contains no nucleotides that do not belong to the binding sequence D of the probe or its complement D'.

APPENDIX C

Marked-up Version showing Amendments to the Claims

1. (Three times amended) A method for the detection of a nucleic acid comprising:
 - (a)- producing a plurality of amplicates of a section of the nucleic acid with the aid of two primers, one of which can bind to a binding sequence [A] A', which is essentially complementary to a sequence A of one strand of the nucleic acid, and the other can bind to a binding sequence C [C' which is essentially complementary to a sequence C] which is located in the 3' direction from A and does not overlap A; and
 - (b)- contacting the amplicates with a probe having a binding sequence D which can bind to a sequence B located between the sequences A and C or to the complement thereof; and
 - (c)- detecting the formation of a hybrid of the amplicate and probe, wherein the sequence between the binding sequences A and C contains no nucleotides that do not belong to a sequence E of the amplicate that is bound by binding sequence D of the probe and the amplicate does not exceed a total length of 100 nucleotides.

5. (Twice amended) The method of claim 1, wherein the total length of the amplicates does not exceed [61] 60 nucleotides.

APPENDIX D

Claims Pending Following Entry of Present Amendment

1. (Three times amended) A method for the detection of a nucleic acid comprising:
 - (a)- producing a plurality of amplicates of a section of the nucleic acid with the aid of two primers, one of which can bind to a binding sequence A', which is essentially complementary to a sequence A of one strand of the nucleic acid, and the other can bind to a binding sequence C which is located in the 3' direction from A and does not overlap A; and
 - (b)- contacting the amplicates with a probe having a binding sequence D which can bind to a sequence B located between the sequences A and C or to the complement thereof; and
 - (c)- detecting the formation of a hybrid of the amplicate and probe, wherein the sequence between the binding sequences A and C contains no nucleotides that do not belong to a sequence E of the amplicate that is bound by binding sequence D of the probe and the amplicate does not exceed a total length of 100 nucleotides.
2. (Previously amended) The method of claim 1, wherein the binding sequence D of the probe overlaps one or both binding sequences of the primers.
3. (Previously amended) The method of claim 1, wherein at least one of the primers has nucleotides in its non-extendible part which do not hybridize directly with the nucleic acid to be detected or with its complement.
4. (Previously amended) The method of claim 1, wherein at least one of the binding sequences is not specific for the nucleic acid to be detected.
5. (Twice amended) The method of claim 1, wherein the total length of the amplicates does not exceed 60 nucleotides.
6. (Previously twice amended) The method of claim 1, wherein at least one of the primers is immobilizably-labeled and the probe is detectably-labeled.
7. (Previously twice amended) The method of claim 1, wherein at least one of the primers is detectably-labeled and the probe is immobilizably-labeled or is immobilized.
8. (Previously twice amended) The method of claim 1, wherein the probe is labeled with a fluorescence quencher as well as with a fluorescent dye.

9. (Previously twice amended) The method of claim 1, wherein one of the primers labeled with a first energy transfer component and the probe is labeled with a second energy transfer component which is different from the first energy transfer component.
10. (Previously amended) The method of claim 1, wherein the amplificate is detected by physical and/or spectroscopic methods.
11. (Previously amended) The method of claim 1, wherein at least one of the primers is not specific for the nucleic acid to be detected.
12. (Previously amended) The method of claim 11, wherein two of the primers are not specific for the nucleic acid to be detected.
13. (Previously amended) The method of claim 11, wherein the probe is not specific for the nucleic acid to be detected.
14. (Previously amended) The method of claim 1, wherein nucleotides which are each complementary to A, G, C and T are used in the amplification.
15. (Previously amended) The method of claim 1, wherein the amplificates are detected by means of mass spectroscopy.
16. (Previously amended) A method for the specific detection of a nucleic acid comprising the steps:
 - (a) - producing a plurality of amplificates of a section of the nucleic acid with the aid of at least two primers,
 - (b) - contacting the amplificates with a probe which can bind to the amplificate, and
 - (c) - detecting the formation of a hybrid of the amplificate and the probe,wherein at least one of the primers is not specific for the group of organisms to which the organism to be detected belongs and the total length of the amplificate does not exceed 100 base pairs.
17. (Previously amended) The method of claim 16, wherein two of the primers are not specific for the nucleic acid to be detected.
18. (Previously amended) The method of claim 16, wherein the probe is not specific for the nucleic acid to be detected.

19. (Previously amended) The method of claim 16, wherein nucleotides which are each complementary to A, G, C and T are used in the amplification.